STIM:Orai stoichiometry and the trapping and activation of store-operated calcium channels

P.J. Hoover and R.S. Lewis , Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305, USA. (Introduced by Grigori Rychkov)

The activation of store-operated Ca^{2+} entry (SOCE) by depletion of Ca^{2+} stores results from the redistribution of the ER Ca^{2+} sensor STIM1 and the CRAC channel protein Orai1 to ER-plasma membrane (PM) junctions where they form closely apposed clusters. Recent studies support a two-part diffusion-trap model for this process, in which the C-terminal polybasic domain of STIM1 binds to phosphoinositides in the junctional plasma membrane and the STIM1 CRAC activation domain (CAD) binds to Orai1, effectively trapping and activating mobile CRAC channels. Store depletion-induced oligomerization of STIM1 has emerged as the essential trigger for this sequence of events, as shown by the ability of artificial STIM1 crosslinking to elicit clustering at junctions and activate CRAC channels in the absence of store depletion. STIM1 traps and activates CRAC channels through the binding of the CRAC activation domain (CAD, aa 342-448) to the N- and C-termini of Orai1.

Crosslinking of individual CRAC channels by the isolated CAD protein fragment suggests that each channel probably contains four STIM binding sites. To determine the minimum number of binding events required to trap a CRAC channel at the ER-PM junction, we measured the junctional ratio of STIM1 to Orai1 as the expression level of Orai1 was increased relative to that of STIM1 in HEK 293 cells. At high Orai1 expression, the STIM:Orai ratio reached a minimum value of ~0.3, or 1.2 STIM/tetrameric CRAC channel, suggesting that a single STIM1 is capable of arresting a CRAC channel at the junction. To determine how CRAC channel activity varies with the number of binding sites occupied by STIM1, we measured I_{CRAC} density in HEK cells expressing a constant amount of STIM1 and increasing levels of Orai1. We found that CRAC channel activation is a highly nonlinear bell-shaped function of Orai1 expression, and that the minimum stoichiometry sufficient for trapping the channels at junctions fails to evoke significant activation. A simple cooperative gating model fitted to the data suggests that only CRAC channels with 4 sites occupied contribute significant current. This highly nonlinear activation of CRAC channels supports earlier conclusions based on current noise analysis (Prakriya & Lewis, 2006) that the slow development of whole-cell CRAC current after store depletion reflects the stepwise recruitment of individual channels from a silent to a high open-probability state as they enter ER-PM junctional sites.

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